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(19)

(54) APPARATUS AND METHOD FOR INNOCULATION

(71) We, THE OPTO ELECTRONIC DISPLAYS LIMITED, of 269a Haydons Road, Wimbledon, London S.W. 19. A British Company, do hereby declare the invention for which we pray that a patent may be granted to us, and the method by which it is performed to be particularly described in and by the following statement: —

This invention relates to an apparatus and method useful for the testing of anti-biotics for their effectiveness against pathogenic micro-organisms.

In conventional medical practice the effectiveness of a particular anti-biotic against a pathogenic micro-organism frequently has to be determined. This can be carried out by innoculating a range of anti-biotics at different concentrations with a liquid containing the pathogenic micro-organism. This process involves the careful addition of a measured amount of liquid to a very large number of individual containers, as the amount of liquid added is small, of the order of 0.05ml, this process can be tedious and time-consuming and opportunities can easily arise for error.

We have now devised an apparatus and method for innoculating anti-biotics with liquids in which less individual innoculation of anti-biotic samples by an operator is required.

According to the invention there is provided an innoculation plate, which plate comprises a plurality of innoculating lines each line comprising a reservoir and a plurality of wells connected by conduits so as to form a continuous liquid flow path from the reservoir sequentially through each well; each well extending in depth below the level of the conduit leading from it at the side distant from the reservoir hereinafter called the exit conduit, the volume of each well beneath the exit conduit being substantially the same, and there being a receiver for excess liquid connected to the well most remote from the reservoir in an innoculating line so as to receive liquid passing out of this well.

The innoculation plate may further comprise a plate support means having a sloping portion whereby when the plate support means is placed on a level surface the sloping portion thereof is at a predetermined angle, whereby when the plate is placed on the sloping portion of the plate support means on a level surface each well can hold a limited pre-determined volume of liquid. By level surface is meant a surface that is horizontal as determined by a spirit level.

The invention further provides a method of innoculating a plurality of wells containing an anti-biotic in dry form using a plate, which plate comprising a plurality of innoculating lines each line comprising a reservoir and a plurality of wells containing an anti-biotic in dry form connected by conduits so as to form a continuous path from the reservoir sequentially through each well, the reservoirs being at the same end of each line, each well extending in depth lower than the conduit leading from it on the side distant from the reservoir, which method comprises placing an excess of innoculating liquid in the reservoir and then placing the plate at a pre-determined angle with the reservoirs at the highest level of each line so that innoculating liquid passes from the reservoirs into each well so that each well contains a limited pre-determined volume of innoculating liquid the excess liquid being collected in a receiver. In the plate the innoculating lines are preferably parallel, the conduits are preferably channels, and wells are upwardly open.

The plate is preferably made of a transparent plastics material, e.g. a polystyrene, a perspex or a p.v.c. (Perspex is a Registered Trade Mark). The plate can then con-

veniently be made from a mould. The wells preferably have a circular cross-section and a conduit enters and leaves each well at opposite ends of a diameter of the circle, more preferably the conduits all lie upon a line passing through the centre of each well. Preferably each well has parallel sides and the conduits enter and leave the well a part way up the side of the well.

Suitable plates comprise from 2 to 12 innoculating lines and each line comprises 4 to 16 wells, a typical plate comprises 96 wells in eight lines of twelve wells. The wells and conduits are preferably arranged so that when the plate is at an angle of e.g. 30° to the horizontal each well can hold from .10-.025ml. of liquid. Preferably there are no sharp corners in the path from the reservoirs through the wells which can interfere with the smooth flow of liquid.

The reservoirs for each innoculating line are preferably formed by dividing a common reservoir by means of baffles, the spaces between the baffles forming the reservoir for each innoculating line, the common reservoir is only partially subdivided by the baffles so that the ends of the reservoirs not connected to the innoculating line open to a common channel.

The reservoirs preferably are upwardly sloping at their end distant from the conduit leaving them for ease of outlet from the reservoirs.

There can be a receiver in each line for excess liquid connected to the well most remote from the reservoir to receive liquid passing out of this well, the receivers for all the lines can optionally be a common receiver.

The plate can be any shape but is preferably rectangular for ease of handling and manufacture.

In a method of the invention by excess amount of innoculating liquid is meant more than enough innoculating liquid to fill each well with the predetermined amount of liquid. The principle of the method lies in the fact that when the plate is at a given angle to the horizontal each well will hold up to a certain volume of liquid before liquid flows out of the conduit on the side distant from the reservoir thus a means of innoculating each well with the same volume of liquid is provided.

In use individual lines can contain varying dosages of the same anti-biotic in different wells and each line can contain a different anti-biotic, preferably one well in each line contains no anti-biotic.

The apparatus of the invention can also be used for innoculating a multi-well plate, for this purpose the wells in the innoculation plate each have a capillary hole formed in them at their lowest surface.

The hole can be positioned anywhere in the lower surface of the well, though preferably it is positioned off-centre of the well.

Each row of wells in the innoculating plate is connected to a reservoir and interconnected so that liquid can be passed from the reservoir to all the wells in the row, and the holes are preferably positioned in the wells at the front of the wells taking the side of each well nearest the reservoir as the front.

Preferably the innoculating plate has a cover which can be heat sealed over it at its edges so that when the wells contain liquid air pressure can be applied to all the wells to force out liquid in each well through the capillary holes.

If desired the cover and the innoculating plate can be connected so that the cover can be folded over the innoculating plate, the cover and innoculating plate can be vacuum-formed from a common sheet of transparent plastics material.

It has been found that when the reservoirs are filled with liquid and the innoculating plate tilted the wells are filled with liquid and the liquid will not flow out through the capillary holes till air pressure is applied to the liquid in the wells. The surface tension of the liquid holds the liquid in the holes till a sufficient air pressure is applied to overcome this effect.

In operation the innoculating unit with the cover heat sealed over it is placed over a multi-well plate with each well in the innoculating plate corresponding to a well in the multi-well plate, liquid applied to each of the reservoirs at the head of each row of wells. The unit is tilted to cause the wells to be filled with liquid. Air pressure is applied to the liquid in the wells in the innoculating unit which forces liquid through the capillary holes in the innoculating unit wells into the wells in the multi-well plate.

When the reservoir for each innoculating line is formed by dividing a common reservoir by means of baffles, and the individual reservoirs are connected by a common channel then the reservoirs are preferably filled by filling the reservoirs with liquid, tilting the plate to cause the liquid to flow into the common channel and thus equally distribute the liquid between the reservoirs. The plate is then tilted to cause the liquid to flow down the innoculating lines and thus fill the wells.

In an application of the invention the method and apparatus of the invention can be used to innoculate a multi-well plate with a plurality of wells having tests for various enzymes and a plurality of wells having pre-selected antibiotic combination in them.

The tests for enzymes can be any selected from a standard range of enzyme tests,

suitable tests include the ONPG, arginine dihydroloase, lysine decarboxylase, ornithine decarboxylase, urease, deaminase, and tests in which enzymes affect a substrate or reagent such as a nitrate, hydrogen sulphide trytophan, indole, acetoin or gelatin, and fermentation tests of glucose, mannitol, inositol, sorbitol, rhaminose, sucrose, melicitose, amygdalin or aribinose. The antibiotic combinations are preferably selected to give an optium result on a range of micro-organisms. In normal operations the species of micro-organism being identified will broadly be known and the anti-biotic combination accordingly chosen.

In operation the sample of material being tested is placed in each of the wells of the plate. In the wells containing the enzyme tests the results of the tests will identify the micro-organism. In the wells containing the antibiotic combination a cell should produce a culture of the pure micro-organism. By noting the colour change combination in the wells containing the enzyme tests and thus identifying the micro-organism it is possible to have a pre-knowledge of which of the anti-biotic containing cells will contain a culture of a single micro-organism. The feasibility of early reading of the colour information could give enough time so that the organisms are still in the logarithmic stage of growth, this could help in the second stage of the antibiotic M.I.C. determination.

A sample from the cell containing the single micro-organism culture can be then used for re-innoculation.

The growth of the micro-organism in the cells containing the anti-biotic combinations can be detected by purely visual means or by detection of fluorescence by exposing the micro-organism to electromagnetic radiation of a wave length at which it will fluoresce if it is growing, and detecting electro-magnetic radiation of a longer wavelength generated by the fluorescence.

A feature of this use of the invention is that it enables one plate to be used for identification of several organisms. For example up to 16 organisms can be tested in a plate of eighty wells by placing the organism in five wells containing different antibiotics and if it is killed in four the fifth well will contain the pure organism.

Another feature of the invention is that the plates, after use, can be readily disposed of, thus reducing the risk from pathogenic organisms.

The invention will now be described with reference to the accompanying drawings in which

Fig. 1 is a plan view of an embodiment of the invention in which the plate is used as a self-innoculating plate.

Fig. 2 is a view along line A-A of fig. 1.

Fig. 3 is a view along line B-B of fig. 2.

Fig. 4 is an enlarged view of part of fig. 2.

Fig. 5 is an enlarged view of fig. 2 in use.

Fig. 6 shows in diagrammatic form the plate in us .

Fig. 7 is a plan vi w of a second embodiment of the invention in which there are capillary holes in the wells in the innoculating plate.

Fig. 8 is a perspective view of the assembled unit and cover shown in Fig. 7.

Referring to fig. 1 a transparent plastic block 1 has circular wells 2 formed in it, which wells 2 are connected to each other by conduits 3. At one end are reservoirs 4, and there is a continuous passage from reservoirs 4 through all the wells in each respective line of wells. At the other end of the conduits is a receiver 5.

Referring to figure 2 each well 2 has a conduit 3 entering and leaving it above its lowermost point.

In use of the embodiment shown in Fig. 1 the plate with a predetermined amount of anti-biotic in each well is placed on portion 10 of plate support means 9 (figure 6). The liquid containing a pathogenic micro-organism is placed in reservoirs 4 in an amount more than sufficient to fill all the wells up to their conduit level connected to that reservoir. The plate is then placed on portion 11 of the plate support means 9 and the liquid runs out of reservoir 4 and flows sequentially through the walls to the receiver 5. Each well is then filled with the liquid 6 as shown in fig. 5.

Thus simply and easily a series of wells can be filled with the same volume of a liquid. The amount of liquid contained in each well will naturally vary depending on the angle the plate is filled to and the material used.

Referring to figs. 7 and 8 an innoculating unit 21 has wells 22 formed in it, only some of the wells are shown. The plate 21 is made from relatively thin plastic and has capillary holes formed in it. Each row of wells are connected to reservoirs 23 and 24, the reservoirs 23 being separated by baffles 30 so that all the reservoirs are connected to common channel. The holes in wells 22 are positioned off-centre nearer the reservoirs 23.

There is a cover 25 connected to plate 21 so that it can be folded over along line 26-26 and heat sealed around the edges to form an air-tight cover as shown in Fig. 8.

In operation the unit 21 and its heat-sealed cover 25 is placed over the multi-well plate to be innoculated and liquid placed in reservoirs 23 through opening 27 in the cover. The reservoirs 23 are separated by baffles 30 to ensure that each row of wells 22, will receive a supply of liquid

and enable the unit 21 to be filled in a single operation. The reservoirs 23 are innoculated with liquid and the plate tilted so that liquid runs into common channel 29 so that the liquid equally distributes itself between the reservoirs 23 separated by baffles 30. The unit is then tilted to cause the liquid to flow from reservoirs 23 down each row of wells 22 so as to fill the wells. The hole 27 is then covered and air pressure applied to the liquid in the wells 22 e.g. by squeezing the top of cover 25. Liquid is forced through the capillary holes in wells 22, and, because the holes are off-centre of the wells liquid comes into contact with the sides of the wells in the multi-well plate and is transferred into the wells into the multi-well plate by surface tension.

The unit 21 and cover 25 can be made of a transparent polyvinyl chloride and shaped by vacuum-forming.

WHAT WE CLAIM IS:

1. An innoculation plate, which plate comprises a plurality of innoculating lines, each line comprising a reservoir and a plurality of wells connected by conduits so as to form a continuous liquid flow path from the reservoir sequentially through each well in the innoculating line; each well extending in depth below the level of the conduit leading from it at the side distant from the reservoir, the volume of each well below the exit conduit being substantially the same and there being a receiver for excess liquid connected to the well most remote from a reservoir in an innoculating line, so as to receive liquid passing out of this well.

2. An innoculation plate as claimed in claim 1 or 2 in which the wells have a circular cross-section and the conduits lie on a horizontal line passing through the centre of each well.

3. An innoculating plate as claimed in any one of claims 1 or 2 which comprises 2 to 12 innoculating lines each containing 4 to 16 wells.

4. An innoculation plate as claimed in claim 1 in which further comprises a plate support means having a sloping portion whereby when the plate support means is placed, on a level surface the sloping portion thereof is at a predetermined angle, to the horizontal whereby when the plate is placed on the sloping portion of the plate support means placed on a level surface each well fills pre-determined volume of liquid.

5. An innoculation plate as claimed in any one of claims 1-4 in which the conduit enters a well a part way up the side of the well.

6. An innoculation plate as claimed in claim 5 which, when the plate is at an angle to the horizontal each well can hold the same volume of liquid in the range of 0.1 to 0.025ml. of liquid.

7. A method of innoculating a plurality of wells containing an anti-biotic in dry form using a plate, which plate comprising a plurality of innoculating lines each line comprising a reservoir and a plurality of wells containing an anti-biotic in dry form connected by conduits so as to form a continuous path from the reservoir sequentially through each well, the reservoirs being at the same end of each line, each well extending in depth lower than the conduit leading from it on the side distant from the reservoir, which method comprises placing an excess of innoculating liquid in the reservoir and then placing the plate at a pre-determined angle with the reservoirs at the highest level of each line so that innoculating liquid passes from the reservoirs into each well so that each well contains a pre-determined volume of innoculating liquid, the excess liquid being collected in a receiver.

8. An innoculating plate as claimed in any one of claims 1-6 in which a plurality of wells contain enzyme *tests* and a plurality of other wells contain a growth medium and a pre-selected anti-biotic combination.

9. An innoculating plate as claimed in claim 8 in which the enzmye tests are selected from ONPG, arginine dihydrolase, lysine decarboxylase, ornithine decarboxylase, urease, and tests in which enzymes affect a substrate or reagent such as a nitrate, hydrogen sulphide, trytophan, indole, acetoin, gelatin, and fermentation tests of glucose, mannitol, inositol, sorbitol, rhaminose, sucrose, melicitose, amygdalin or aribinose.

10. An innoculating plate as claimed in any one of claim 1-3 in which the wells have a capillary hole formed in them at their lowest position.

11. An innoculating plate as claimed in claim 10 which has a cover, which can be heat sealed over it, so that when the wells contain liquid, air pressure can be applied to all the wells to force out liquid in each well through the capillary holes.

12. An innoculating plate as claimed in claim 11 in which the cover and innoculating plate are formed from a transparent sheet of material and the cover can be folded over the innoculating plate.

13. An innoculating plate as claimed in any one of claims 10-12 in which the reservoir for each innoculating line is formed by the separation of a common reservoir by means of baffles so as to form a reservoir for each innoculating line, the reservoirs being connected to a common channel at their ends not connected to the in-

noculating line.

14. A method of innoculating a multi-well plate which comprises placing an innoculating plate as claimed in any one of claim 10-13 over the multi-well plate so that the wells in the innoculating plate correspond to wells in the multi-well plate, for putting liquid in the reservoirs in the innoculating plate, tilting the plate to fill the wells in the innoculating plate with liquid and applying air pressure to the wells in the innoculating plate to force the liquid through the holes.

15. A method as claimed in claim 14 in which the reservoirs are connected to a common channel at their end not connected to the innoculating lines, and after putting liquid in the reservoirs, the innoculating plate is tilted to fill the common channel with liquid and to distribute liquid equally between the reservoirs and then the innoculating plate is tilted to fill the wells in the innoculating plate with liquid.

16. An innoculating plate as hereinbefore described with reference to figures 1-6 of the accompanying drawings.

17. An innoculating plate as hereinbefore described with reference to figures 7 and 8 of the drawings.

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Agent for the Applicants.

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1572596 COMPLETE SPECIFICATION

3 SHEETS *This drawing is a reproduction of the Original on a reduced scale*

Sheet 1

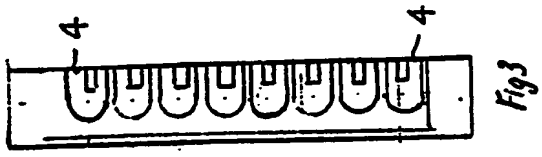

Fig3

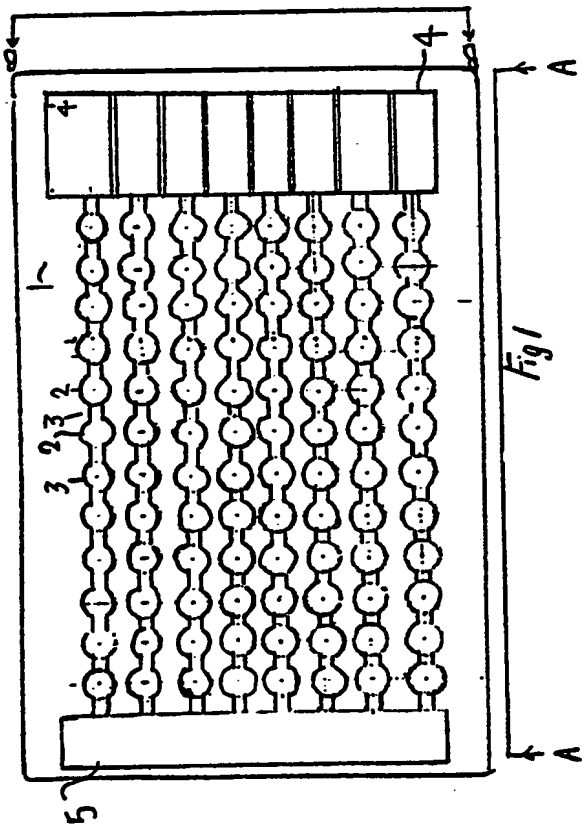

Fig1

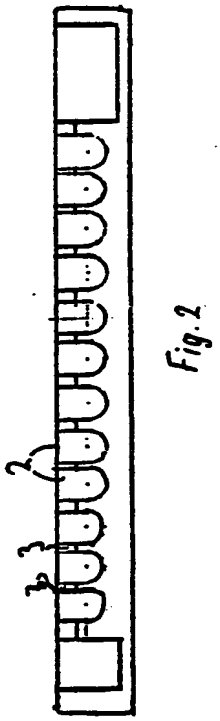

Fig.2

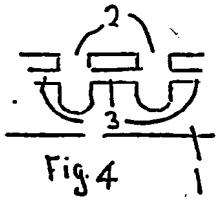

Fig.4

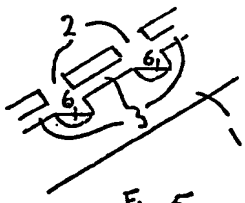

Fig.5

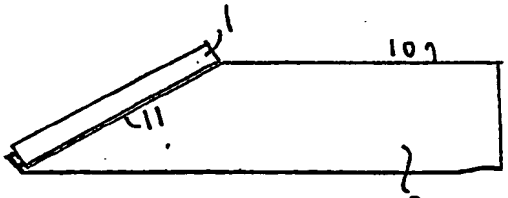

Fig.6

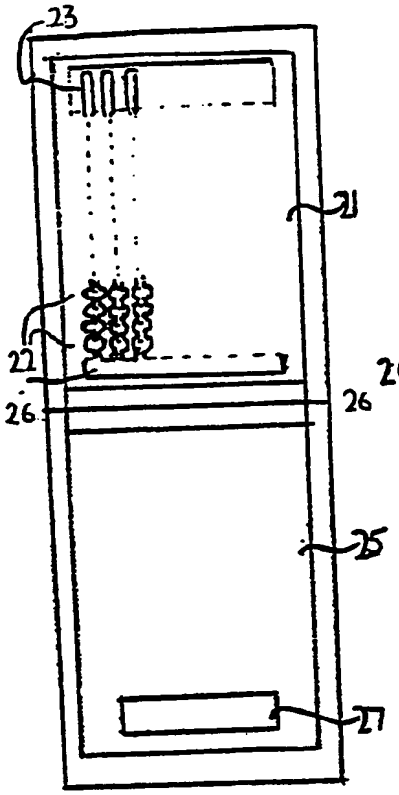

Fig1

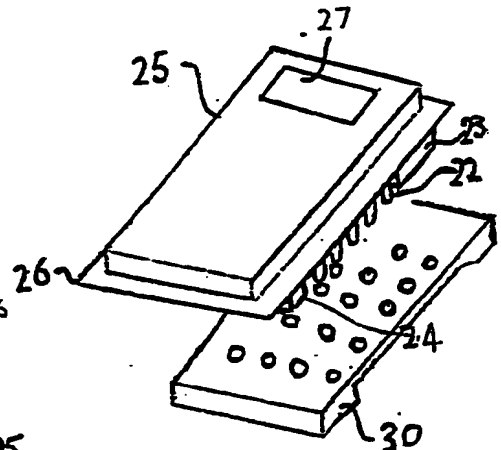

Fig.8